

Amendments to the Claims/Listing of Claims

Please amend claims 19, 28, 31, 38, 42, 43 and 46 as follows. This listing of claims will replace all prior versions, and listings, of claims in the application:

1. – 18. (Cancelled)

19. (Currently amended) A method for minimizing or eliminating injury to a living system **otherwise** caused by cooling said living system without the formation of ice therein, said method comprising:

adding to said living system a preservation medium having a tonicity which is sufficiently hypertonic to minimize injury caused by said cooling, wherein said preservation medium has a tonicity between 1 and 4 times isotonic, and wherein said preservation medium comprises a carrier solution and at least one cryoprotective agent which is sufficient in concentration to prevent freezing at a predetermined temperature below approximately 0°C; and

cooling the living system to said predetermined temperature, thereby minimizing or eliminating injury caused by said cooling.

20. (Cancelled)

21. (Previously presented) The method of Claim 19, wherein the tonicity is from 1.1 to 2.7 times isotonic.

22. (Previously presented) The method of Claim 19, wherein the tonicity is from 1.1 to 2 times isotonic.

23. (Previously presented) The method of Claim 19, wherein the tonicity is from 1.1 to 1.5 times isotonic.

24. (Previously presented) The method of Claim 19, wherein the tonicity is from 1.2 to 1.5 times isotonic.

25. (Previously presented) The method of Claim 19, wherein the tonicity of the preservation medium is increased by increasing the concentration of said carrier solution.

26. (Previously presented) The method of Claim 25, wherein the tonicity of the carrier solution is raised simultaneously with an increase in concentration of said cryoprotective agent.

27. (Previously presented) The method of Claim 26, wherein the tonicity of the carrier solution is increased by a proportion which is approximately equal to the proportional increase in the concentration of said cryoprotective agent.

28. (Currently amended) The method of Claim 27, further comprising reducing the tonicity of the preservation medium ~~to isotonic~~ while the concentration of said cryoprotective agent is diluted in similar proportion to the dilution of the carrier solution.

29. – 30. (Cancelled)

31. (Currently amended) A method for minimizing or eliminating injury to a living system ~~otherwise~~ caused by cooling said living system without the formation of ice therein, said method comprising:

adding to said living system a preservation medium having a tonicity which is sufficiently hypertonic to minimize injury caused by said cooling, wherein said preservation medium has a tonicity between 1 and 4 times isotonic, and wherein said preservation medium comprises a carrier solution, at least one cryoprotective agent, and at least one polymer, wherein said cryoprotective agent and said polymer are sufficient in concentration to prevent freezing at a predetermined temperature below approximately 0°C; and

cooling the living system to said predetermined temperature, thereby minimizing or eliminating injury caused by said cooling.

32. (Previously presented) The method of Claim 31, wherein the at least one polymer is selected from the group consisting of polyglycerol, polyvinyl alcohol-polyvinyl acetate copolymer, polyvinyl pyrrolidone, polyethylene glycol, and mixtures thereof.

33. (Cancelled)

34. (Previously presented) The method of claim 32 wherein said polyethylene glycol has a mean molecular mass of approximately 1000 daltons.

35. (Previously presented) The method of Claim 19, wherein said at least one cryoprotective agent comprises dimethyl sulfoxide, formamide, and ethylene glycol, and optionally further comprises acetol.

36. (Previously presented) The method of Claim 35, wherein said at least one cryoprotective agent further comprises polyglycerol and polyvinyl alcohol-polyvinyl acetate copolymer, and wherein the combination of polyglycerol and polyvinyl alcohol-polyvinyl acetate copolymer is at a total concentration of 0.1 to 0.7 times isotonic.

37. (Cancelled)

38. (Currently Amended) A method for minimizing or eliminating injury to a living system ~~otherwise~~ caused by cooling said living system without the formation of ice therein, said method comprising:

contacting said living system with a first protective solution prepared by adjusting the hypertonicity of the solution to be within a first tonicity range for minimizing injury caused by said cooling within a first predetermined temperature range, wherein said first protective solution has a tonicity between 1 and 4 times isotonic;

cooling the living system to a temperature within said first temperature range;

contacting said living system with a second protective solution prepared by adjusting the hypertonicity of the solution to be within a second tonicity range for minimizing injury caused by

said cooling within a second predetermined temperature range, wherein said second protective solution has a tonicity between 1 and 4 times isotonic; and

cooling the living system to a temperature within said second temperature range, thereby minimizing or eliminating injury caused by said cooling.

39. (Previously presented) The method of Claim 38, wherein said first protective solution has a hypertonicity lower than the hypertonicity of said second protective solution.

40. (Previously presented) The method of Claim 38, wherein said first protective solution contains at least one antinucleating polymer.

41. (Previously presented) The method of Claim 38, wherein said second protective solution contains at least one antinucleating polymer.

42. (Currently amended) The method of Claim 38, wherein said first protective solution contains polyvinyl pyrrolidone ~~having a mean molecular mass of approximately 5000 daltons.~~

43. (Currently amended) The method of Claim 38, wherein said second protective solution contains polyvinyl pyrrolidone ~~having a mean molecular mass of approximately 5000 daltons.~~

44. (Previously presented) The method of Claim 38, wherein said first protective solution contains polyethylene glycol having a mean molecular mass of approximately 1000 daltons.

45. (Previously presented) The method of Claim 38, wherein said second protective solution contains polyethylene glycol having a mean molecular mass of approximately 1000 daltons.

46. (Currently amended) A method for minimizing or eliminating injury to a living system ~~otherwise~~ caused by cooling said living system without the formation of ice therein, said

method comprising:

adding to said living system a preservation medium having a tonicity within an optimum tonicity range for minimizing or eliminating injury **otherwise** caused by cooling said living system to a predetermined temperature below approximately 0°C without the formation of ice therein; wherein said optimum tonicity range is between 1 and 4 times isotonic, and wherein said preservation medium comprises a carrier solution and at least one cryoprotective agent which is sufficient in concentration to prevent freezing during said cooling to said predetermined temperature below approximately 0°C; and

cooling the living system to said predetermined temperature, thereby minimizing or eliminating injury **otherwise** caused by said cooling.